J. Appl. Environ. Biol. Sci., 1(7)126-128, 2011

ISSN 2090 – 424X
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Biomanagement of Banana Leaf Waste through Microbial Technology

T. Srinivasan* and G. S. Vijayalakshmi

Department of Environmental Sciences, SPKCES, Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tamil Nadu, India.

ABSTRACT: Banana agrowaste, dried leaves create solid waste pollution problem after usage. In the present study, these wastes were used as substrate for the production of cellulolytic enzymes with *Trichoderma viridae* (MTCC 1763). The banana leaves as carbon source gave good results with the enzyme activity of 0.27 U/ml FPase, 0.46 U/m1 CMCase, and 0.32 U/m1 β-glucosidase. In enzyme assay optimization study, pH 5.5 of the medium was found as optimum whereas 45°C was optimum temperature. The saccharification of banana leaf wastes by cellulases of *Trichoderma viridae* (MTCC 1763) was also investigated. The steam subjected banana leaf wastes yielded 1.36 mg/ml of reducing sugar after 24 h at 5.5 pH and 45°C.

Keywords: Banana leaf waste, Cellulases, *Trichoderma viridae*, Saccharification.

INTRODUCTION

Crop residue management in agriculture for sustaining high levels of crop productivity is the biggest challenge for researchers. Cellulose, a major constituent of agricultural residues can be degraded by a wide range of microorganisms. In plants cellulose usually found in association with hemi-cellulose and lignin as a component of lignocellulose. The enzymatic hydrolysis of cellulose takes place under the action of cellulase complex containing endoglucanase, exoglucanase and glucosidase. These enzymes comprise together a system to convert cellulose to glucose [1]. India is largest producer of banana, contributing to 27% of world's banana production. Tamil Nadu is the leading producer of banana, followed by Maharashtra. Banana plant has an origin from India and eastern Asian region (Malayasia and Japan). Most productive cultivars are Cavendish banana and giant French plantains (Productive value, >30 t /ha / yr) [2]. Out of over 50 varieties of banana cultivated across India, 20 varieties are commonly grown.

The bioconversion of agro waste based lignocellulosic material to energy has gained much interest during the recent past. Low cost of enzyme production improves the economics, as the cost of enzymes constitutes a major part of the total cost of hydrolysis [3]. The enzymatic degradation of waste cellulose by fungal enzymes has been suggested as a feasible alternative for the conversion of lingo cellulosic material in to fermentable sugars and ethanol [4, 5]. After harvest of banana crop, the leaves left in the field for natural degradation, which takes several months. Besides, banana leaf wastes thrown out in huge amount from hotels, markets, human residential areas during festival seasons create pollution problems. Hence, in the present study, this agrobased waste, i.e., banana leaf wastes has been utilized for the production of cellulases and in saccharification to produce reducing sugar with the help of *Trichoderma viridae* (MTCC 1763) [6].

MATERIALS AND METHODS

The banana leaf wastes were collected from hotels, market, marriage halls and municipal waste heaps in and around Kovilpatti of Tuticorin District, Tamilnadu. The collected leaves were washed thoroughly with water, dried in the oven at 70°C. The material was finely powdered (40 mesh–120 μ) and used as substrate for the production of cellulases. Delignification of the material was done by treating l0g of dried powder in 500ml of 0.1N NaOH at 121°C for 20 minutes in an autoclave and powder was supplemented to the basal medium.

Trichoderma viride MTCC 1763 was procured from Microbial Type Culture Collection and Gene bank, Institute of Microbial Technology (IMTECH), Chandigarh, India. *T. viridae* was maintained on Potato Dextrose Agar (PDA) slants and stored at 4 ± 1°C and subcultured fortnightly. *T. viridae* was transferred from agar slants to Petri plates containing PDA and incubated at room temperature for 10 days. After delignification, the powder of banana leaf wastes was supplemented to the basal medium. Sterilized 50ml of liquid substrate was taken in 250ml Erlenmeyer flasks and inoculated with 5 mycelial disc (7mm dia) punched out from the edges of its 8th day old colonies in Petri plates. The flasks were incubated at 27±2°C for 10 days. The culture filtrate was collected, centrifuged at 2000g for 30 minutes to remove all spores. The supernatant was filtered through what man No.1 and was dialysed against running tap water for 24h. This preparation was used as crude cellulolytic enzyme solution during the course of study.

***Correspondence Author:** T. Srinivasan, Department of Environmental Sciences, SPKCES, Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tamil Nadu, India. E-mail: tsvnbiotech@yahoo.com

The activity of filter paper cellulase (FPase) was assayed following recommendations of IUPAC [7] using Whatman No.1 filter paper as substrate. Carboxymethyl cellulose enzymes (CMC ase) was assayed using 1% CMC solution as substrate and activity of β-glucosidase activity was determined [8]. For saccharification the substrate was inoculated with 5ml of culture filtrate obtained from 8th day of inoculation of *T. viridae* MTCC 1763 in enzyme assay studies and reducing sugar was assayed using DNS method [8]. The release of sugar is expressed as equivalent to glucose.

RESULTS AND DISCUSSION

The analysis of banana leaf waste showed 28.5, 24.57 and 9.7% of cellulose, hemicellulose and lignin respectively. The carbon source in the medium affects considerably in the synthesis of the cellulolytic enzymes by *T. viridae MTCC 1763* in liquid cultures. The present study shows that the optimum pH for production of FPase, CMCase and β-glucosidase by *T. viridae* in banana leaf waste based medium was 5.5 and optimum incubation temperature was 45±2°C (Tables 1 and 2).

Acidic pH was favourable for the enzyme production, whereas the enzyme production substantially decreased at pH 6.5. The previous research findings reveal that 45°C was the optimum temperature for cellulase activity [9, 10, 11]. Among different pH range from 3.5 to 6.5 in 8th day of incubation at 45±2°C showed maximum cellulase activities in terms of CMC-ase, FPase, and β-glucosidase were 0.46, 0.18 and 0.37 U/ml respectively. The degree of saccharification was assayed on the basis of release of reducing group. The amount of reducing sugars increased with time of incubation in the presence of enzyme. In 12 h of incubation there was 0.64 mg/ml of reducing sugar released and the maximum amount of reducing sugar was released at the end of 24 h was 1.36mg/ml. The saccharification of banana leaf wastes by crude enzyme produced by *T. viride* MTCC 1763 indicates enzymes acts on the substrate specifically to release reducing sugar which in turn produce alcohol if further fermentation takes place.

Table 1. Effect of incubation period on cellulase production from banana leaves waste in different days of interval and pH optimization in 27 ±2°C after inoculation of *T. viridae MTCC 1763.*

	Period of incubation														
	2 Day			4 Day			6 Day			8 Day			10 Day		
pH	CMC	FP -ase	B-Glu	CMC	FP -ase	B-Glu	CMC	FP -ase	B-Glu	CMC	FP -ase	B-Glu	CMC	FP -ase	B-Glu
3.5	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.02	0.06	0.13	0.03	0.09	0.08	0.02	0.06
4.5	0.02	0.00	0.00	0.07	0.00	0.01	0.18	0.08	0.12	0.26	0.09	0.17	0.23	0.08	0.14
5.5	0.09	0.00	0.02	0.28	0.03	0.16	0.28	0.1	0.18	0.41	0.15	0.25	0.38	0.13	0.22
6.5	0.02	0.00	0.08	0.12	0.00	0.07	0.17	0.06	0.09	0.25	0.07	0.16	0.13	0.00	0.04

Table 2. Effect of temperature on cellulase production from banana leaf wastes in different days of interval at pH 5.5 after inoculation of *T. viridae* MTCC 1763.

	Period of incubation														
	2 Day			4 Day			6 Day			8 Day			10 Day		
Temp. (°C)	CMC	FP ase	B-Glu	CMC	FP ase	B-Glu	CMC	FP ase	B-Glu	CMC	FP ase	B-Glu	CMC	FP ase	B-Glu
25	0.00	0.00	0.00	0.04	0.00	0.01	0.13	0.08	0.07	0.25	0.14	0.16	0.21	0.13	0.15
35	0.06	0.00	0.01	0.13	0.02	0.06	0.24	0.11	0.12	0.35	0.18	0.21	0.32	0.16	0.2
45	0.09	0.00	0.02	0.18	0.03	0.07	0.31	0.13	0.19	0.46	0.27	0.32	0.42	0.16	0.29
55	0.07	0.00	0.00	0.16	0.02	0.05	0.28	0.11	0.16	0.44	0.17	0.3	0.4	0.15	0.23

ACKNOWLEDGEMENTS

The authors gratefully thank the Managing Director, Thiru. K.R. Arunachalam, National Educational Trust and Dr. S. Chockalingam, Principal, K.R. College of Arts & Science, Kovilpatti, Tamil Nadu for their support in carrying out this research work. Thanks are also due to Dr. N. Karmegam, Dept. of Botany, Govt. Arts College, Salem-7, Tamil Nadu for his encouragement.

REFERENCES

[1] Antony, M.V., Mohan, S., Kasinathan, P. and Thajuddin, N. (2004). Production and properties of cellulolytic enzymes by Trichoderma viridae and Trichoderma

harzianum in submerged fermentation. Asian J. Microbiol. Biotech. Env. Sci., 6: 395 – 401.

[2] Ortiz, R. and Vuylsteke, D. (1998). Quantitative variation and phenotypic correlations in banana and plantain. Sci. Hortic., 72: 239 – 253.

[3] Depaula, E.H., Ramos, L. P. and Azevedo, M.D. (1999). The potential of Humicola grisea var. thermoidea for bioconversion of sugarcane bagasse. Bioresour. Technol., 68: 35 – 41.

[4] Shin, C.S., Lee, J.P. and Park, S.C. (2000). Enzyme production of Trichoderma reesei Rut C–30 on various lignocellulosic substrates. Appl. Biochem. Biotechnol., 86: 237–245.

[5] Szengyel, Z., Zacchi, G., Varga, A. and Reczey, K. (2000). Cellulase production of Trichoderma reesei RUT C30 using steam pretreated spruce. Hydrolytic potential of cellulases on different substrate. Appl. Biochem. Biotechnol., 86: 679–691.

[6] Baig, M.M.V., Mane, V.P., More, D.R., Shinde, L.P. and Baig, M.A. (2003). Utilization of agricultural waste of banana: production of cellulases by soil fungi. J. Environ. Biol., 24: 173–176.

[7] Ghose, T.K. (1987). Measurement of cellulase activities. Pure Appl. Chem., 59: 257–268.

[8] Miller, G.L. (1959). Use of dinitro salicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426–429.

[9] Jha, K., Khare, S.K. and Gandhi, A.P. (1995). Solid state fermentation of soy hull for the production of cellulase. Bioresour. Technol., 54: 321–322.

[10] Mukhopadhyay, S. and Nandi, B. (1999). Optimization of cellulase production by Trichoderma reesei ATCC 26921 using a simplified medium on water hyacinth biomass. J. Sci. Ind. Res., 58: 107–111.

[11] Baig, M.M.V. (2005). Cellulolytic enzymes of Trichoderma lignorum produced on banana agro waste: optimization of culture medium and conditions. J. Sci. Indust. Res., 64: 57-60.